THE MICROSCOPIC INNERVATION OF THE URINARY BLADDER IN LOWER VERTEBRATES

by

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Concerning the nerve supply of the urinary bladder in *Anamnia*, especially in frogs, many examinations were made with methylblue staining about the end of the last century (cit. in 8). These examinations gave a general description about the quantity and quality of the cells, however, the question of the endforms could not indisputably be stated. To study the nerve endings the silver impregnation methods are more suitable than the vital staining. With impregnation we got quite different pictures about the innervation of the urinary bladder in frogs as with methyl-blue available in the literary data. We succeeded to impregnate not only the motoric endings but also the pressoreceptors of the urinary bladder in frogs and tortoises which, however, failer in mammals.

Material and method

The material used: the urinary bladder of carps *(Cyprinus carpio)*, frogs *(Rana ridibunda* and *esculenta)*, lizards *(Lacerta agilis* and *taurica)* and tortoises *(Emys orbicularis)*. To have sections from this material only carp's bladder was needed. The bladder wall of the other three animals is so thin that we had to stretch them on a cork-plate with help of *Erinaceus*' thorns and after fixation we could use them as total preparation, very adequate to examine the nerve connections. The fixation fluid was 10% formol or AFA, the impregnation was made with the BIELSCHOWSKY—ÁBRAHÁM's method (2).

Nerve supply of the bladders

The nerve fibres of the urinary bladder belong mostly to sympathetic and parasympathetic system originating from the lower part of the paravertebral trunk. The two kinds of fibres can not be differentiated, both are characterized in the bladder wall by the nerve cells very closely connected to the vegetative trunks. However, not alone these thin vegetative fibres but in a lesser number cerebrospinal (spinal or *vagus* origin) thick fibres occur in the bladder. The different nerve fibres and nerve cells form the microscopic innervation of the bladders in which interesting differences are shown among the examined animals.

Fishes

In the carp which possesses very well developed urinary bladder was found a very rich nerve supply. The elliptic bladder has very thick wall. Its structure differs from that of the frogs and reptiles. Here the smooth muscle layer is quite compact similar to the mammals. The nerve trunks running from the outher connective tissue layer to the smooth muscle bundles form a very rich *plexus* on their surface. The fibres of the *plexus* run always separately and end as very thin end-fibres on the smooth muscle cells. Sometimes little

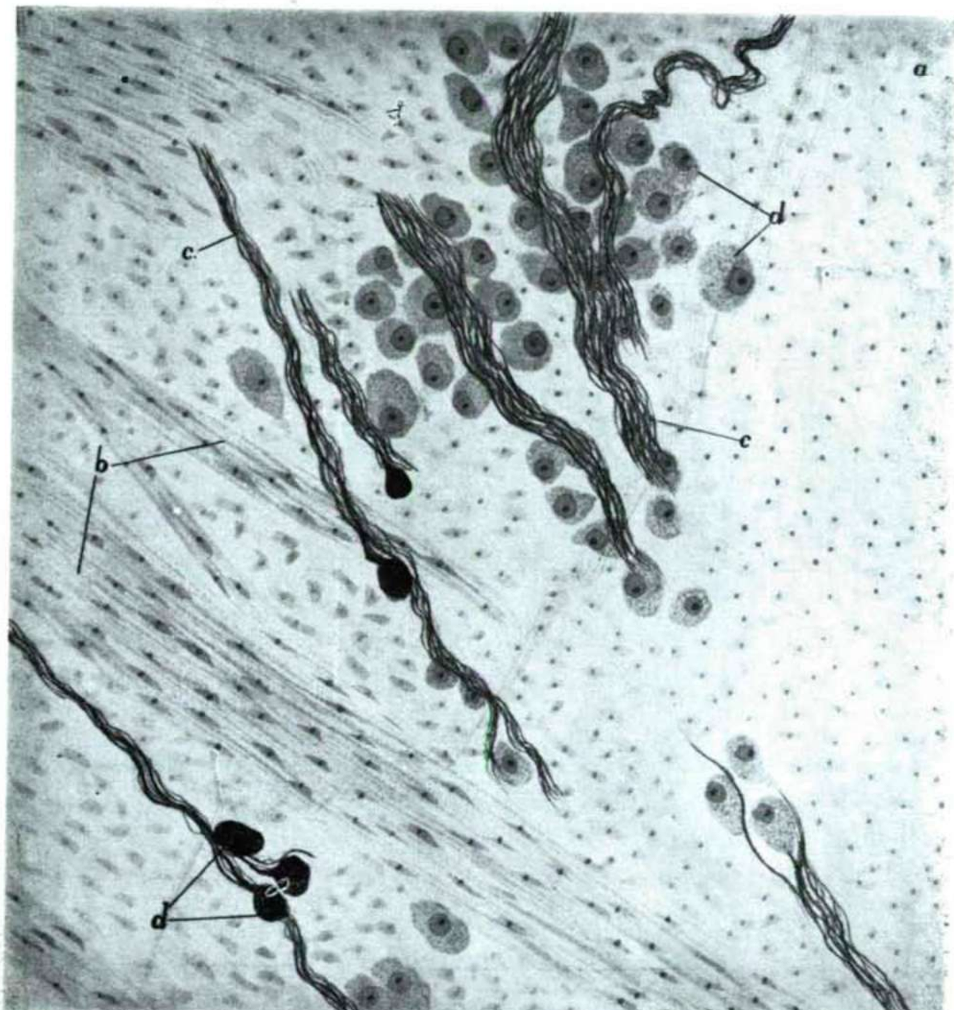

Fig. 1. Nerve cells of different size and colour in the urinary bladder of carp. a-connective tissue; b-smooth muscle bundle; c-nerve trunk; d-nerve cells. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 660×. Photographically reduced to 1/2.

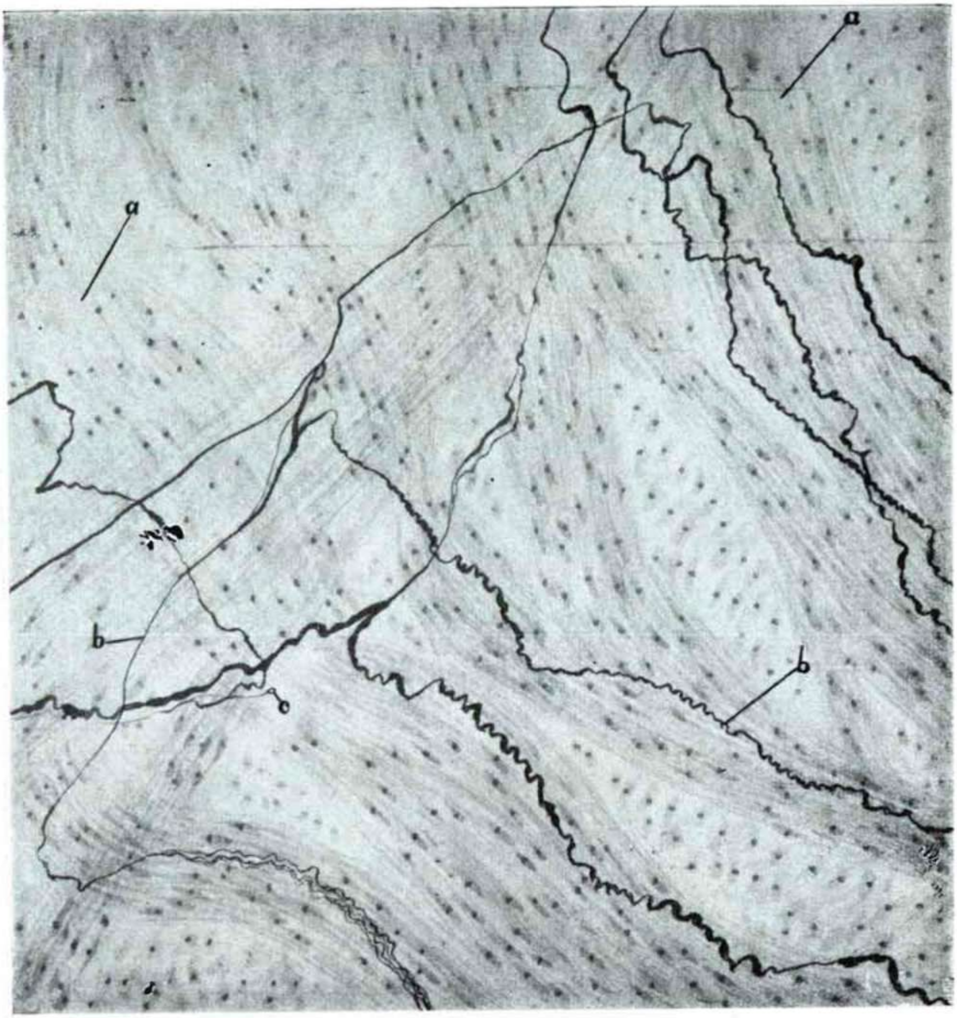

Fig. 2. Innervation of the urinary bladder in frog. a-smooth muscle bundle; b-nerve fibre; c-nerve end. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 660×. Photographically reduced to $^1/_2$.

end-spots may be noted on the end. In the inner epithelial layer of the bladder, no nerve fibres were discernible. The outer layer of the urinary bladder contains numerous blood vessels and very many *ganglia* (Fig. 1). The nerve cells appear not merely in the outer layer but among the smooth muscle bundles. They belong to the uni- and bipolar type. Interesting is that these intramural cells show very large differences in size and they appear in two quite different colours. There are cells of light brown colour continuing in thin processes to innerve the muscle and very many cells of dark black colour with thick nerve processes showing very many varices. Sometimes we can not distinguish whether cells or ends or varices appear on the slide. It is sure, they belong to one system. It may be that this black coloured, very argentophyl nerve cells represent the sensory system of the bladder. If it is so, according to its appearance they forward the stimuli directly from the inner layer to the muscle layer as a proprioreceptive reflex with a single neuron. End-aparatuses never occurred on the cells of carp' urinary bladder.

Frogs

The urinary bladder entirely differs from that of the carp. It originates not on the usual way but from the bulding of the *proctodeum.* Structurally it is so thin a plate as to render possible its total examination. So the three layers are visible together particularly fit to examine nerve connections. On such

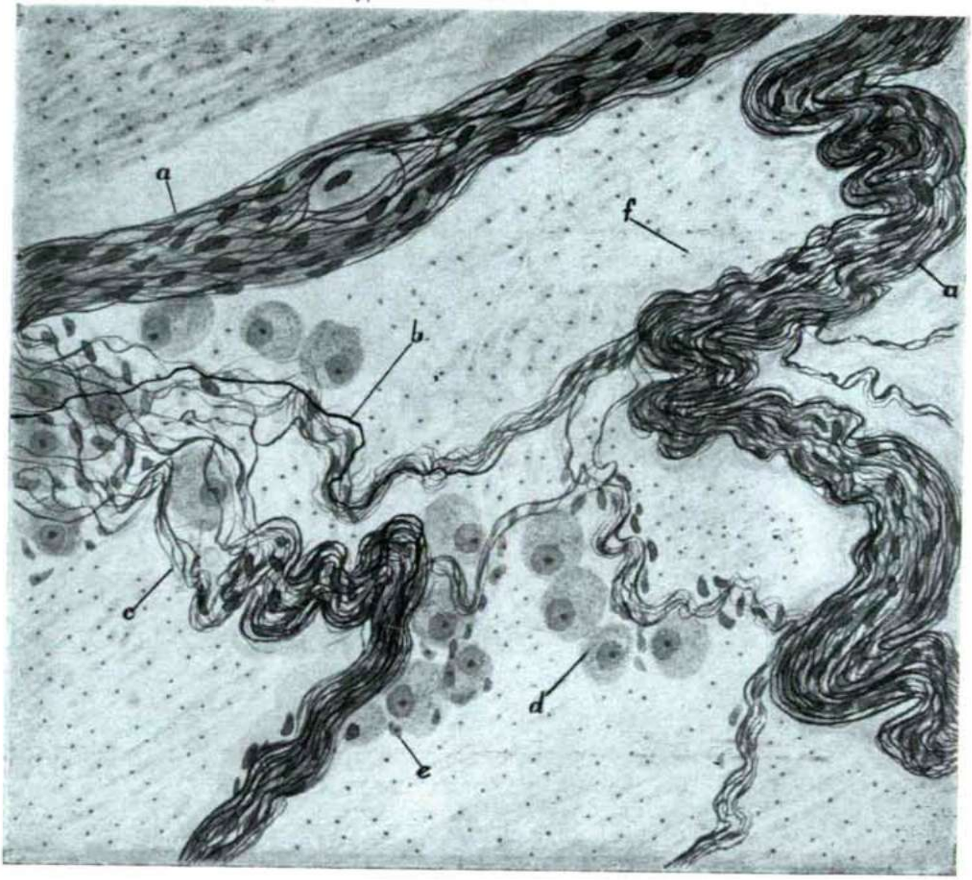

Fig. 3. Nerve cells and trunk connections at the *hylus* of the urinary bladder in frogs. a-nerve trunk; b-thick nerve fibre; c-thin nerve fibre; d-nerve cell; e-nucleus of glia cell; f-nucleus of muscle cell. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 660×. Photographically reduced to $^1/_2$.

a total preparation between the outer and inner layer smooth muscle bundles serve not only to contract but to support. These bundles are connected with each other and form a very loose net in the bladder. This net is strikingly visible with impregnation because the nerve fibres run only on the surface. (Fig. 2). If the bladder wall was well stretched, the nerve fibres are quite

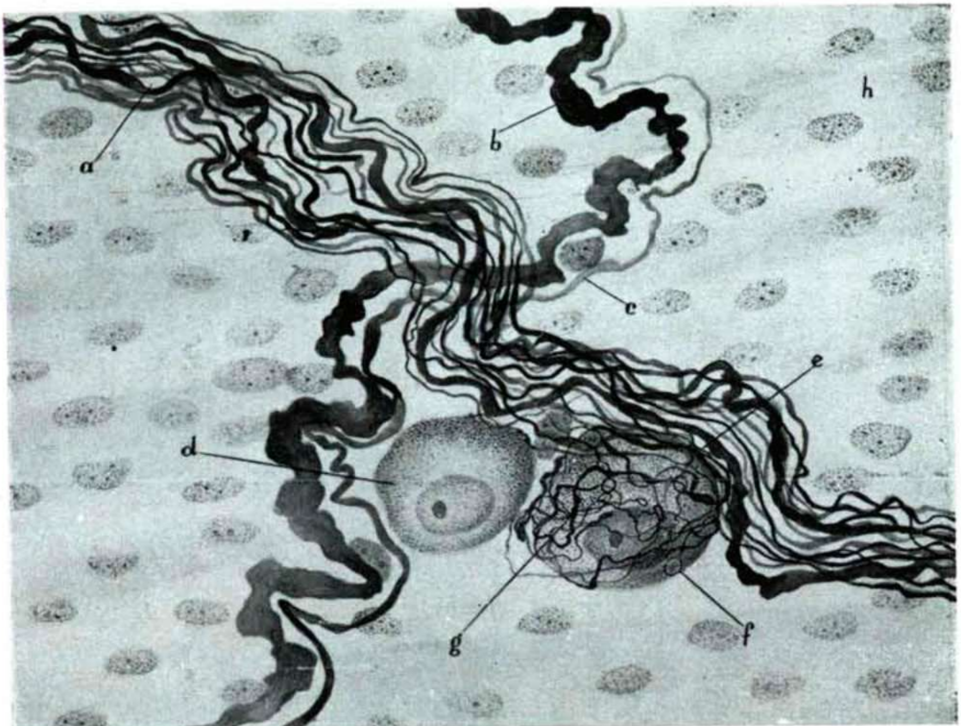

Fig. 4. Synapsis-form (1) in the urinary bladder of frog. a-nerve trunk; b-thick nerve fibre; c-thin nerve fibre; d-nerve cell; e-end fibre; f-end ring; g-pericellular basket; h-connective tissue. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 900×. Photographically reduced to $^1/_2$.

straight or if not they follow the contracted muscle cells in zig-zag. It follows that the nerve trunks of the bladder are in close connection with the smooth muscle cells branching on their surface and ending thereon. The ends occur very frequently in form of rings or spots (Fig. 2. c).

The nerve cells are found in the nerve trunk of vegetative fibres. They often appear in frogs as single cells following each other very densely at the outer side of the trunk. These single cells are always uni- or bipolar sending their one or two processes into the trunk running to innerve the smooth muscle bundles. Sometimes the cells form quite large intramural *ganglia*. These *ganglia* are found at the entrance of the nerve trunks where they branch off (Fig. 3).

The size of the cells is quite identical and among the cells uni-, bi- and multipolar type may be noted. Very many synapses occur on the cells. In SEREBRJAKOW's well-known work six kinds of cells were distinguished — with

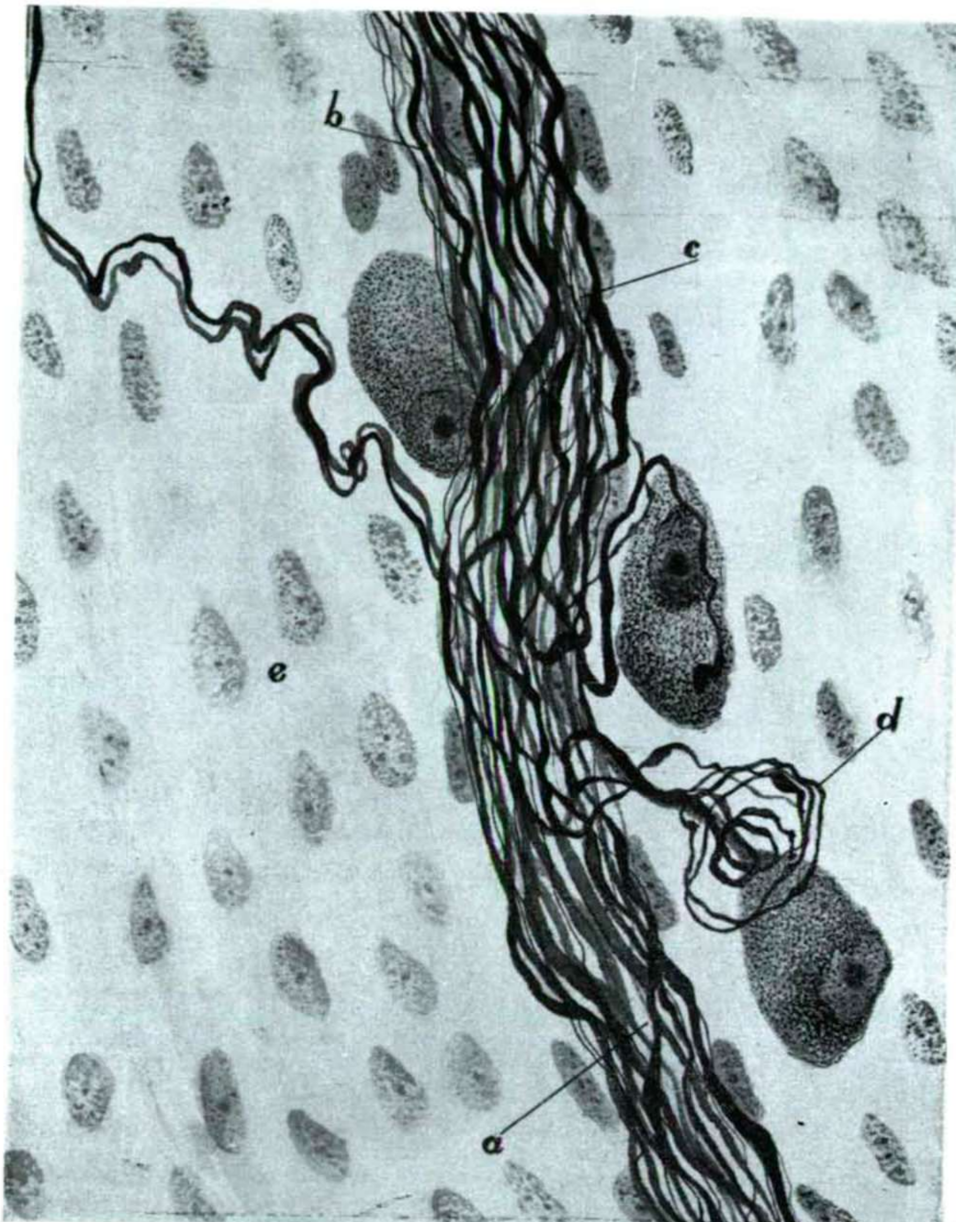

Fig. 5. Synapsis-form (2) in the urinary bladder of frog. a-nerve trunk with cells; b-thick nerve fibre; c-thin nerve fibre; d-end spiral; e-connective tissue. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 900×. Photographically reduced to $^1/_2$.

methyl-blue staining — and pericellular apparatuses were never absent from the vegetative cells in frogs. However these six cell-forms are not so well discernible with our silver impregnation. Neither the six kinds of pericellular ends nor the very varicous fibres on the cell surface could be demonstrated in such forms as SEREBRJAKOW's (8). We, too, found the speciality of the frogs' cells i. e. the end-spirals described by SMIRNOW (9) on all vegetative cells of the bladder. These end-apparatuses are synapses; very interesting and complicated forms of the connections between the cerebrospinal-vegetative and sympathetic-parasympathetic system. There are more differences than six among these cell synapses. Morphologically there are so many synapsis-forms as cells. In our opinion two kinds of pericellular end-apparatuses appear that differ not only structurally but functionally too.

One of the end-forms is a pericellular basket situated all over the surface of the cells. The end-branchings of the end-fibres end in rings or knots penetrating very deeply into the cell body (Fig. 4). It is very difficult to decide whether the ends are in the plasm or on the surface of the cell body. The latter seems to be more accepted, confirmed by the new electron-microscopical data. We think these synapses arising from the cerebrospinal nerve fibres as they are often quite thick at the beginning.

The second type of the synapses are very sharply impregnated spirals visible on the process of the cell. The end fibre is not on the cell surface but ends in a coil around the process. (Fig. 5.) The fibre of the spirals comes always from an other trunk containing very wavy thin fibres and never followed by cells. The nerve cell possessing this spiral belongs to such a trunk that has many *glia* cells among the fibres. We think that these synapses connect the two kinds of vegetative (sympathetic and parasympathetic) system. Concerning the origin of the synaptical fibres we wish to confirm the morphological data with operations in the future.

Reptiles

The microscopic structure of the bladder in lizards and tortoises resembles very much to that of the frogs. The muscular bundles form more compact layer than in the frogs therefore the nerve *plexus* is more extended. The picture of the innervation is in full agreement with that of the frogs. The thin and thick fibres appear here too but the number of the nerve cells is not so high. They are situated in 2—3 large groups but only when entering the nerve trunk, quite close to the *hylus*. Single nerve cells, very characteristic of the frogs, occur very rarely. Remarkable is that the pericellular apparatuses did not appear in such great number as in the frogs. However, they were never found in lizards and rarely enough in tortoises. Their form was the pericellular basket but in considerably simpler forms.

Pressoreceptors in the urinary bladder

In nerve trunks of the urinary bladders thick fibres can be very often noted. Looking for their end it was found that they give very tortuous branches passing from the muscle bundles to the inner layer of the bladder and form a dendron-like ramification at the end. The varicous structure of these fibres is always characteristic and rings or spots may occur at their end. Sometimes 3—4 end-fibres are quite close to each other showing their end ramifications and so a great receptory area is formed (Fig. 6).

These complicated forms were found only in frogs, but the simple ones are quite common not only in frogs but also in lizards and tortoises (Fig. 7).*

Fig. 6. Pressoreceptor in the urinary bladder of frog. a-nerve trunk; b-nerve plexus on the smooth muscle bundle; c-sensory fibre; d-ending; e-connective tissue. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 660×. Photographically reduced to 1/2.

The ramification bears great similarity to the pressoreceptors in the blood vessels, heart wall as well as in the swimm-bladder found by ÁBRAHÁM (1—5). The findings confirm ÁBRAHÁM's opinion that the number of the receptors is constantly growing and becomes more complicated in the highest animals; however the human body has the most differentiated receptors. At any rate it is interesting that the urinary bladder of *Anamnia* possesses these pressoreceptors in great number, but never found them in this form in the bladder of mammals (6, 7, 10).

* The figures were drown by E. DÁNOS and G. MRÁZ technicians of the Institute.

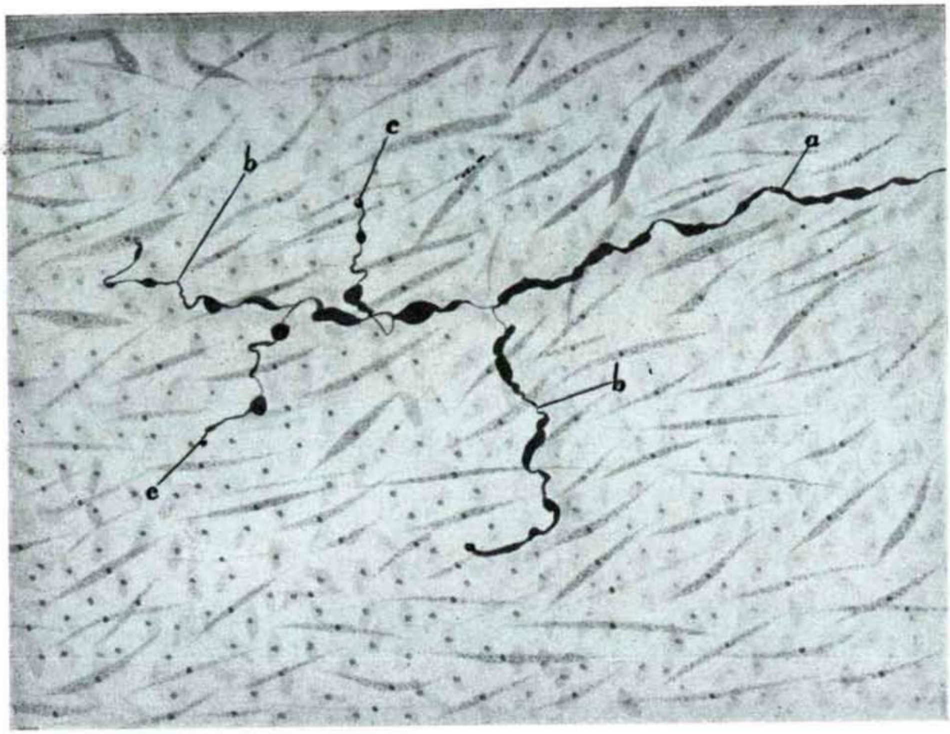

Fig. 7. Pressoreceptor in the urinary bladder of tortoise. a-sensory fibre; b-end fibre; c-ending. BIELSCHOWSKY—ÁBRAHÁM's method. Magnification 900×. Photographically reduced to $^1/_2$.

Summary

Differences were found in the innervation of the urinary bladder of the examined animals. Mainly the nerve cells were variable. The uni- and many bipolar cells of different size in carps are visible in great number in two quite different colours: 1. cells of light brown colour continuing in thin nerve processes to innerve the smooth muscle and 2. cells of dark black colour with thick nerve processes showing very many varices and ending in bulbs close to the *epithelium*. The uni- and multipolar nerve cells in frogs are in groups or as single cells following the fibres and possess always pericellular apparatuses. However the multipolar cells of reptiles are always in groups at the entrance of the bladders and pericellular apparatuses appear only rarely on their surface.

They agree in the very rich innervation of the urinary bladder and in the fact that the thin nerve fibres end in the plexuses of the smooth muscles, wherein the end fibres always remain independent and never form *reticulum*. The thick fibres end with dendron-like ramifications with spots and rings on their end. These ramifications represent the simple form of receptors and according to the similarity and function of other organs they are considered as pressoreceptors.

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